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Voluntary Feed Intake and Egg Quality Traits of Caged Vs Cage-free Layers as Affected by Black Soldier Fly (Hermetia illucens L.) Larvae Supplementation

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Abstract

The study examined the impact of black soldier fly larvae (BSFL) supplementation on the voluntary feed intake (VFI) and egg quality traits of Dekalb White layers in a caged and cage-free housing system. Ninety-six (96) hens were experimentally manipulated according to a 2x3 factorial design, examining the effects of two independent variables: housing system (caged or cage-free) and the proportion of BSFL in the diet (0%, 10%, 20%). Uncaged birds with 0% BSFL showed significantly higher VFI ($p<0.05$). Analysis of egg quality revealed a significant interaction effect ($p < 0.05$) for egg shape index (ESI) only, where caged hens on a 0% BSFL diet attained the maximum value. Yolk weight and yolk color were significantly higher in uncaged hens receiving 20% BSFL ($p < 0.05$). Furthermore, the uncaged housing system independently exhibited significant effects on egg weight, egg height, egg width, shell weight, shell thickness, and albumen weight ($p < 0.05$). The yolk-albumen ratio, however, did not differ significantly across treatments ($p > 0.05$). It was concluded that both housing systems and BSFL levels exert significant effects on the VFI and egg quality traits of layer chickens.

Keywords: black soldier fly larvae, cage-free system, Dekalb White layers, egg quality traits, voluntary feed intake

1. Introduction

Cage-free systems are gaining traction worldwide as driven by consumer concerns regarding animal welfare and product quality. Cage-free housing systems, in contrast to conventional caged systems, provide hens with the opportunity to express natural behaviors, thereby mitigating stress and aggression while promoting enhanced well-being and superior egg quality (Hartcher & Jones, 2017; Senčić et al., 2006). Furthermore, recognizing that free-range birds naturally consume insects while foraging, researchers are exploring the incorporation of insect-based diets for cage-free layers. This approach offers a sustainable alternative to traditional protein sources without compromising egg production or hen health

(Maurer et al., 2016). Insects are emerging as a promising and sustainable component in poultry nutrition due to their high nutrient density and potential to enhance layer performance (Sajid et al., 2023). Notably, Hermetia illucens larvae are rich in protein, essential amino acids (lysine and methionine), fatty acids, and minerals, making them an ideal candidate for poultry feed (Lu et al., 2022). Several studies have demonstrated that the utilization of BSFL in layer chicken diets can enhance egg quality by increasing albumen proportions, reducing cholesterol content, and improving eggshell quality (Mlaga et al., 2022; Nassar et al., 2023). While research on BSFL utilization in broiler nutrition is extensive, there is a relative paucity of literature examining its effects on cage-free laying hens, particularly concerning VFI and egg quality traits. Therefore, this study aimed to comprehensively investigate and address these research gaps.

2. Materials and Methods

Experimental Design

The study involved 96 ready-to-lay Dekalb White hens, assigned to either caged or cage-free environments. A 2x3 factorial design within a randomized complete block design (RCBD) with four blocks was implemented to investigate the effects of housing system (caged or cage-free) and BSFL supplementation (0%, 10%, 20% w/w of the basal diet). This design resulted in six treatment groups (A-caged,0%; B-uncaged,0%; C-caged,10%; D-uncaged,10%; E-caged,20%; F-uncaged,20%), with four randomly assigned hens per treatment within each block.

Site and Diet Preparations

Caged hens were housed at a density of 0.09 m^2 per bird in 12 enclosures (Enos, 2018), while cage-free hens were maintained at a density of 1 m^2 per bird in 12 range areas (Oliveira et al., 2022). Following a seven-day adjustment period, hens received 120 g/bird/day of a commercial layer diet (Ranson, 2023), supplemented with live BSFL according to the assigned treatment level. Live BSFL utilized were at the third to fifth instar developmental stage. The VFI and egg quality parameters were recorded weekly in the 12-week duration of the study.

Statistical Analysis

The collected data were subjected to a two-way analysis of variance (ANOVA) within the framework of a 2x3 factorial arrangement in RCBD. This analysis was conducted using the General Linear Model (GLM) procedure in SAS® OnDemand for Academics. Post-hoc multiple comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to assess significant differences in interaction (A x B) and main effects (A, B). The threshold for statistical significance was set at $\alpha = 0.05$.

3. Results and Discussion

Voluntary Feed Intake

Voluntary feed intake is important for layer hens, impacting their growth, egg production, and health. Table 1 shows VFI for hens in two housing systems supplemented with different BSFL levels over 12 weeks. No significant A x B interaction was found ($p>0.05$), but significant differences in feed intake were observed between housing systems in the early weeks, as well as fluctuations in BSFL supplementation levels at specific time points ($p<0.05$). In terms of housing, significant differences were noted at weeks 2 and 4 (p = 0.0190 and p = 0.0316), with uncaged hens consistently consuming more feed (6.33 kg and 6.47 kg) than caged hens (6.16 kg and 6.24 kg). These results support Saiful et al. (2003) and Tahamtani et al. (2020), who found that uncaged hens exhibit higher feed intake due to increased energy expenditure from more movement. The lack of significant differences in later weeks suggests that hens adapted to their environments, stabilizing their feed intake.

BSFL supplementation showed significant effects on feed intake at weeks 2 (p = 0.0021) and 6 (p = 0.0192). At week 2, hens fed 20% BSFL had the lowest intake (6.18 kg) compared to 10% (6.38 kg) and 0% (6.42 kg), aligning with Facey et al. (2024), who suggested that higher BSFL levels may reduce palatability or alter energy balance. At week 6, intake was lowest in the 10% group (6.29 kg). These patterns may reflect hens' difficulty in choosing between basal feed and larvae, with increased levels complicating selection. While this may lower intake, it also supports natural foraging behavior. Digestibility and nutrient differences in BSFL diets may also contribute (Makkar et al., 2014; Gasco et al., 2019; Kwakernaak, 2023).

Table 1. Voluntary Feed Intake of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	Kg/WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	6.16^{b}	6.24^{b}	6.34	6.45	6.38	6.35	6.32
Uncaged	6.33^{a}	6.47^{a}	6.38	6.28	6.36	6.35	6.36
SEM	0.046	0.068	0.027	0.134	0.019	0.009	0.041
p-VALUE	0.0190	0.0316	0.3405	0.3664	0.3763	0.9462	0.5074
LEVELS							
0%	6.42^{a}	6.46	6.46^{a}	6.48	6.42	6.33	6.42
10%	6.38^{b}	6.48	6.29^{c}	6.43	6.31	6.38	6.37
20%	6.18^{c}	6.46	6.37^{b}	5.90	6.33	6.34	6.26
SEM	0.033	0.041	0.020	0.326	0.035	0.015	0.054
Linear Trend	0.0021	1.000	0.0192	0.2515	0.1059	0.9104	0.0571

Means within a column having different superscripts are statistically significant (p<0.05).

Egg Quality Traits

Egg Weight

Egg weight, a key factor in market value and consumer acceptability, showed no significant A x B interaction (p>0.05). Table 2 results revealed that housing systems significantly influenced egg weight at weeks 4 (p = 0.0129), 10 (p = 0.0003), and 12 (p < 0.0001), with uncaged hens producing heavier eggs. At week 12, uncaged hens' eggs averaged 65.43 g, significantly heavier (p < 0.0001) than the 59.57 g of eggs from caged hens. The overall mean followed the same trend, with uncaged hens at 63.81 g and caged hens at 61.14 g. These findings contrast with Yildirim and Kaya (2017), who reported heavier eggs in indoor systems. The improved physiological conditions of uncaged hens, along with nutritional benefits from their environment (e.g., vegetation, insects, and solar radiation), may explain the difference. No significant egg weight differences were observed between housing systems during weeks 2, 6, and 8 (p > 0.05), suggesting that the impact of housing on egg weight increases as the laying cycle progresses.

Table 2. Egg Weight (grams) of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	62.43	61.40^{b}	61.89	62.41	59.19^{b}	59.57^{b}	61.14^{b}
Uncaged	63.00	63.78^{a}	62.89	64.55	63.26^{a}	65.43^{a}	63.81^{a}
SEM	0.978	0.595	1.070	0.949	0.622	0.629	0.573
p-VALUE	0.6863	0.0129	0.5192	0.1302	0.0003	<0.0001	0.0216
LEVELS							
0%	63.45	64.75	64.12	66.71	62.95	65.66	64.60
10%	61.04	62.33	61.66	63.12	64.62	66.91	63.28
20%	65.50	64.25	62.87	63.83	62.20	63.71	63.72
SEM	2.184	0.871	1.913	1.761	0.923	1.380	0.661
Linear Trend	0.7473	0.6691	0.6605	0.2916	0.5865	0.3548	0.3693

Means within a column having different superscripts are statistically significant ($p<0.05$).

BSFL supplementation had no significant effect on egg weight across all weeks, including the overall mean ($p > 0.05$). This contrasts with Wamai et al. (2024), who found increased egg weight with 75% BSFL inclusion, suggesting that egg weight may be less responsive to insect meal in moderate amounts. Onsongo et al. (2018) noted that longer trials (over 20 weeks) may be needed to detect significant effects. Hence, a 12-week period in this study might not have been long enough to observe significant physiological changes. The temporary rise in egg weight at week 2 (65.50 g) in the 20% BSFL group may be linked to the higher lipid content of BSFL, which supports yolk formation (Thao et al., 2023).

Egg Height and Width

Egg height, an important indicator of egg quality and albumen freshness (Stadelman, 2017), is presented in Table 3. No significant A x B interaction was found ($p>0.05$), but housing systems significantly influenced egg height at weeks 4 ($p = 0.0364$), 8 ($p = 0.0265$), 10 ($p< 0.0001$), 12 ($p<0.0001$), and the overall mean ($p = 0.0477$). Uncaged hens produced eggs with greater height (60.23 mm) compared to caged hens (58.60 mm), supporting Alig et al. (2023), who observed heavier eggs in uncaged systems. Keshavarz and Nakajima (1995) attributed this to more varied diets, including forage or insects, which boost protein, vitamin D, and calcium intake—key for eggshell quality. Increased mobility in uncaged systems may also enhance calcium utilization, promoting stronger, more elongated eggs (Hood & Hobensack, 2015).

Table 3. Egg Height (millimeters) of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	59.72	58.99^{b}	59.39	58.61^{b}	57.59^{b}	57.35^{b}	58.60^{b}

Uncaged	59.79	60.39[a]	59.47	59.96[a]	60.68[a]	61.11[a]	60.23[a]
SEM	0.509	0.431	0.409	0.386	0.323	0.395	0.440
p-VALUE	0.9247	0.0364	0.8897	0.0265	<0.0001	<0.0001	0.0477
LEVELS							
0%	60.00	62.00	59.83	60.70	60.79	60.54	60.64
10%	59.08	59.87	59.58	59.83	60.83	61.87	60.17
20%	60.29	59.29	58.99	59.33	60.41	60.91	59.87
SEM	1.048	0.918	0.779	0.912	0.418	0.872	0.300
Linear Trend	0.8501	0.0823	0.4773	0.3277	0.5497	0.7729	0.0993

Means within a column having different superscripts are statistically significant (p<0.05).

BSFL supplementation had no significant effect on egg height (p>0.05), suggesting minimal influence from dietary changes. This aligns with Franco et al. (2020), who found egg height is primarily influenced by genetic and environmental factors rather than diet. The lack of variation across treatments indicates that 10–20% BSFL inclusion does not affect albumen structure or internal egg quality, possibly due to the similar amino acid profile and digestibility of BSFL compared to the control diet (Maurer et al., 2016).

Table 4 presents the effects of housing systems and BSFL supplementation on egg width. Egg width is important for shape index, influencing consumer appeal and storage. The housing system had a significant effect at weeks 4 (p = 0.0164), 8 (p = 0.0336), and 12 (p < 0.0001), with uncaged hens producing significantly wider eggs than caged hens. By week 12, egg width averaged 43.74 mm for uncaged hens versus 41.77 mm for caged hens (p < 0.0001). This supports findings by Dikme et al. (2016), who linked greater egg size to enhanced musculoskeletal development in uncaged birds. Increased activity in these systems may also improve calcium metabolism, benefiting egg formation (Fu et al., 2022). Additionally, reduced stress in uncaged environments, as noted by Campbell et al. (2019), may support better hormonal regulation and egg development.

Table 4. Egg Width (millimeters) of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	44.07	42.56[b]	43.24	42.75[b]	42.57	41.77[b]	42.82[b]
Uncaged	44.21	43.45[a]	43.75	43.58[a]	43.02	43.74[a]	43.62[a]
SEM	0.331	0.236	0.314	0.251	0.500	0.238	0.183
p-VALUE	0.7734	0.0164	0.2657	0.0336	0.5399	<0.0001	0.0276
LEVELS							
0%	45.04	43.87	44.42	44.37	43.66	43.21	44.09
10%	43.66	43.37	43.62	43.08	43.75	44.16	43.60
20%	43.91	43.08	43.20	43.29	41.62	43.83	43.32
SEM	0.610	0.481	0.588	0.4424	1.229	0.544	0.303
Linear Trend	0.2413	0.2885	0.1949	0.1343	0.2852	0.4493	0.1016

Means within a column having different superscripts are statistically significant ($p<0.05$).

BSFL supplementation had no significant effect on egg width throughout the study ($p > 0.05$), indicating that dietary insect meal does not notably influence lateral eggshell development. This aligns with Franco et al. (2020), who emphasized the stronger role of genetics and environment over diet. The lack of effect may also relate to BSFL's chitin content, which can support gut health but potentially reduce protein digestibility when present in excess (Borrelli et al., 2017). These findings support Al-Qazzaz et al. (2016), suggesting BSFL inclusion up to 20% does not negatively impact egg morphology.

Egg Shape Index

Table 5 presents egg shape index (ESI) data across treatment groups over the 12-week trial. ESI, the ratio of egg width to length, is optimal between 0.72 and 0.76 (Adeolu & Oleforuh-Okoleh, 2021). A significant interaction between housing and diet (A × B) was observed ($p < 0.05$), though no significant differences appeared in early weeks ($p > 0.05$). By week 12 ($p = 0.0396$) and in the overall average ($p = 0.0113$), differences became evident. Caged hens without BSFL (Treatment A) had the highest ESI (0.73), while uncaged hens with 10% BSFL (Treatment D) had the lowest (0.66). The overall mean revealed that Treatments A and C (caged with 0% and 10% BSFL) had higher ESIs (0.71 and 0.72), while uncaged hens with 20% BSFL (Treatment F) had the lowest (0.68), suggesting housing and diet interact to influence egg shape.

Table 5. Egg Shape Index of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

WEEK	TREATMENT COMBINATION						SEM	p-VALUE
	A	B	C	D	E	F		
2	0.72	0.72	0.73	0.74	0.70	0.69	0.020	0.8149
4	0.71	0.68	0.72	0.70	0.68	0.67	0.012	0.7413
6	0.70	0.69	0.72	0.72	0.72	0.69	0.019	0.8417
8	0.69	0.67	0.71	0.69	0.67	0.68	0.014	0.5669
10	0.73	0.70	0.73	0.68	0.73	0.67	0.012	0.6558
12	0.73^{a}	0.67^{c}	0.70^{b}	0.66^{c}	0.71^{ab}	0.70^{b}	0.007	0.0396
AVERAGE	0.71^{a}	0.69^{b}	0.72^{a}	0.70^{ab}	0.70^{ab}	0.68^{b}	0.007	0.0113

Legend: A- caged with 0% BSFL, B- uncaged with 0% BSFL, C- caged with 10% BSFL, D- uncaged with
10% BSFL, E- caged with 20% BSFL, F- uncaged with 20% BSFL.
Means within a row having different superscripts are statistically significant ($p<0.05$).

Previous studies have shown that eggs from caged hens typically have slightly higher shape index values (Svobodová et al., 2014; Galic et al., 2019; Rodríguez-Mengod et al., 2024), likely due to restricted movement, which reduces physical stress on the reproductive system and promotes uniform egg shape (Hartcher & Jones 2017). In contrast, increased activity and environmental stress in uncaged systems may cause slight shape irregularities (Lay et al., 2011). This may explain the lower egg shape index observed in uncaged hens receiving 20% BSFL (Treatment F) compared to caged hens.

BSFL inclusion at 10-20% did not consistently improve the egg shape index (ESI). Hens in caged systems with 10% BSFL supplementation (Treatment C) showed a relatively high ESI, but no clear trend emerged across different supplementation levels. This supports previous findings that ESI is largely influenced by genetic factors and housing conditions rather than diet alone (Tougan & Thewis, 2024). The results suggest that caged systems may produce eggs with a slightly higher shape index, while uncaged systems with higher BSFL levels might yield lower ESI values. This could be due to the hens' adaptive mechanisms. Jehl et al (2019) noted that layers adjust feed intake to meet nutrient needs, potentially minimizing performance differences across treatments.

Shell Weight and Thickness

According to Li et al. (2024), eggshell quality traits, such as strength and thickness, are crucial for commercial purposes, influencing egg durability and marketability. Shell weight, a significant indicator of eggshell strength, is primarily influenced by calcium metabolism and dietary composition (Jiang et al., 2013).

Table 6 data showed no significant interaction effects between factors A and B ($p>0.05$). However, housing systems significantly influenced shell weight at weeks 10 ($p < 0.0001$) and 12 ($p < 0.0001$), as well as in the overall mean ($p = 0.0277$). Hens in uncaged systems produced eggs with heavier shells (week 12: 6.44 g vs. caged 5.71 g; overall mean: 6.52 g vs. caged 6.17 g), consistent with Lichovníková and Zeman (2018), who attributed this to enhanced calcium utilization due to increased movement and bone mineralization.

Table 6. Shell Weight (grams) of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	6.01	6.42	7.15	6.25	5.53^{b}	5.71^{b}	6.17^{b}
Uncaged	6.21	6.54	7.25	6.52	6.18^{a}	6.44^{a}	6.52^{a}
SEM	0.102	0.092	0.107	0.091	0.065	0.085	0.079
p-VALUE	0.1999	0.3659	0.5351	0.0584	<0.0001	<0.0001	0.0277
LEVELS							
0%	6.29	6.62	7.33	6.66	6.12	6.20	6.53
10%	6.12	6.62	7.24	6.54	6.24	6.41	6.52
20%	6.21	6.37	7.16	6.33	6.16	6.71	6.49
SEM	0.203	0.209	0.104	0.178	0.089	0.212	0.065
Linear Trend	0.7845	0.4304	0.2927	0.2367	0.7615	0.1457	0.6256

Means within a column having different superscripts are statistically significant ($p<0.05$).

BSFL supplementation had no significant effect on shell weight ($p>0.05$), though hens receiving 0% BSFL had the heaviest shells (6.53 g), while those with 10% and 20% BSFL had lighter shells (6.52 g and 6.49 g, respectively). This suggests that insect-based proteins might influence calcium absorption and shell formation (Gautron et al., 2021), though the 12-week duration may not have captured significant differences across supplementation levels.

Table 7 presents the eggshell thickness means over the 12-week study period. No significant interaction effect was observed (p>0.05). However, the housing system significantly influenced eggshell thickness (p < 0.05), with uncaged hens consistently producing thicker shells than caged hens, particularly at weeks 2, 4, 6, 8, 10, and 12 (p-values <0.0001 to 0.0287). At week 12, uncaged hens had an average thickness of 0.37 mm, compared to 0.34 mm in caged hens. This aligns with Galic et al. (2019), who linked increased physical activity in uncaged systems to better calcium mobilization for eggshell formation.

Table 7. Shell Thickness (millimeters) of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	0.34^b	0.35^b	0.34^b	0.33^b	0.33^b	0.34^b	0.33^b
Uncaged	0.37^a	0.38^a	0.38^a	0.38^a	0.38^a	0.37^a	0.37^a
SEM	0.009	0.004	0.006	0.004	0.005	0.005	0.002
p-VALUE	0.0287	<0.0001	0.0005	<0.0001	<0.0001	0.0013	0.0002
LEVELS							
0%	0.36	0.37	0.36	0.37	0.37	0.36	0.36
10%	0.39	0.38	0.39	0.39	0.40	0.36	0.38
20%	0.37	0.39	0.37	0.37	0.38	0.37	0.37
SEM	0.013	0.010	0.013	0.006	0.011	0.008	0.003
Linear Trend	0.6296	0.1119	0.6296	0.6149	0.6738	0.4351	0.0707

Means within a column having different superscripts are statistically significant (p<0.05).

BSFL supplementation had no significant effect on shell thickness (p>0.05), despite marginal increases at 10% BSFL. This suggests the insect meal's limited impact on calcium metabolism when hens receive a balanced diet with adequate calcium (Negoiță et al., 2018). Previous studies indicate that calcium, phosphorus, and vitamin D3 are more influential on eggshell thickness than dietary protein sources alone (Shi et al., 2020), supporting the idea that BSFL supplementation does not affect shell thickness in the presence of sufficient calcium.

Yolk and Albumen Weight

Table 8 presents yolk weight data for eggs produced in caged and uncaged systems with varying BSFL levels. No significant A x B interaction was found (p>0.05). However, housing systems significantly influenced yolk weight, with uncaged hens consistently producing heavier yolks than caged hens (p<0.05). Significant differences were observed at weeks 2 (p = 0.0150), 6 (p = 0.0410), 10 (p = 0.0102), and 12 (p < 0.0001), with uncaged hens having heavier yolks, especially at week 12. These findings align with Rodríguez-Hernández et al (2024), who attributed heavier yolk weights in cage-free systems to unimpeded movement and more favorable conditions for yolk development.

Table 8. Yolk Weight (grams) of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	19.00^b	18.60	19.49^b	20.01	19.74^b	19.00^b	19.30^b
Uncaged	21.09^a	19.50	21.08^a	21.14	21.28^a	21.28^a	20.89^a
SEM	0.538	0.346	0.505	0.428	0.371	0.179	0.153
p-VALUE	0.0150	0.0854	0.0410	0.0830	0.0102	<0.0001	0.0008
LEVELS							
0%	20.08	19.04	21.00	21.29	20.46	21.54	20.56^b
10%	20.81	19.75	20.29	21.04	21.16	21.04	20.68^b
20%	22.37	19.70	21.96	21.08	22.21	21.25	21.42^a
SEM	0.730	0.528	0.849	0.927	0.925	0.287	0.243
Linear Trend	0.0684	0.4062	0.4548	0.8794	0.2297	0.5022	0.0314

Means within a column having different superscripts are statistically significant (p<0.05).

Sherwin et al (2010) suggested that greater freedom of movement and more comfortable laying postures also contribute. BSFL supplementation had no significant impact on yolk weight (p>0.05), except for the overall mean (p = 0.0314), where 20% BSFL led to a higher yolk weight. This suggests that insect inclusion does not significantly affect yolk weight when hens receive a balanced diet. Previous studies have indicated that yolk weight is more influenced by lipid sources, energy intake, and genetics than by insect protein (Thao et al., 2023; Tougan & Thewis, 2024; Franco et al., 2023). The type of fatty acids in BSFL and their metabolism may not have significantly influenced yolk deposition (Wu et al., 2020).

Table 9 presents albumen weight data. No significant interaction effect was observed (p>0.05), but significant differences were found in housing systems, particularly at weeks 4 (p = 0.0236), 10 (p = 0.0017), 12 (p <0.0001), and the overall mean (p = 0.0248). Uncaged hens produced heavier albumen than caged hens, especially at week 12, with uncaged hens averaging 41.43 g compared to 37.31 g in caged hens. This is consistent with Thao et al. (2023), who linked increased albumen weight to physical activity and protein metabolism in cage-free systems.

Table 9. Albumen Weight (grams) of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	37.81	38.57^b	38.40	40.14	37.36^b	37.31^b	38.26^b
Uncaged	37.78	40.43^a	39.32	41.85	40.82^a	41.43^a	40.27^a
SEM	0.678	0.522	0.887	0.725	0.639	0.467	0.447
p-VALUE	0.9768	0.0236	0.4765	0.1170	0.0017	<0.0001	0.0248
LEVELS							
0%	37.54	41.54	39.79	43.16	41.50	41.33	40.81

10%	37.33	40.62	38.66	40.41	40.91	42.37	40.05
20%	38.45	39.12	39.50	41.95	40.04	40.58	39.94
SEM	1.539	0.877	1.798	1.618	1.136	0.855	0.369
Linear Trend	0.6888	0.0994	0.9130	0.6167	0.4003	0.5609	0.1267

Means within a column having different superscripts are statistically significant ($p<0.05$).

Campbell et al (2019) also noted that uncaged hens had lower stress and better albumen weight. BSFL supplementation had no significant effect on albumen weight ($p>0.05$), likely due to adequate protein in the diet. This contrasts with studies linking high-protein diets to improved albumen quality (Gunawardana et al., 2009), possibly due to differences in BSFL nutrient balance, anti-nutritional factors (Borrelli et al., 2017), or hen genetics and environment (Marono et al., 2017).

Yolk-to-Albumen Ratio

The yolk-to-albumen (Y:A) ratio is an important indicator of egg composition and nutritional quality, as it reflects the distribution of dry matter (lipids and proteins) within the egg (Islam et al., 2017).

No significant A x B interaction was observed ($p>0.05$). The ratios in Table 10 showed no significant differences between housing systems ($p>0.05$), indicating consistent Y:A ratios in both caged and uncaged systems. Although marginal differences were noted, uncaged hens had a slightly higher Y:A ratio, particularly at week 6 (0.54), compared to caged hens (0.51). While studies compare egg quality between cage-free and conventional systems, none specifically report a higher Y:A ratio in cage-free systems.

BSFL supplementation had no significant effect on the Y:A ratio ($p>0.05$) across all weeks. This suggests that insect meal inclusion did not enhance yolk deposition, despite BSFL's lipid content and contribution to yolk formation. These results contradict Liu et al. (2021) and Thao et al. (2023), who found that alternative protein sources rich in bioavailable lipids positively influence yolk development without affecting albumen quality.

Table 10. Yolk-to-Albumen Ratio of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	0.52	0.48	0.51	0.50	0.53	0.51	0.50
Uncaged	0.55	0.48	0.54	0.51	0.52	0.52	0.52
SEM	0.018	0.010	0.017	0.005	0.009	0.009	0.004
p-VALUE	0.1701	0.8644	0.2583	0.4488	0.5610	0.4155	0.1345
LEVELS							
0%	0.56	0.46	0.53	0.49	0.49	0.52	0.50
10%	0.50	0.48	0.54	0.52	0.51	0.51	0.51
20%	0.58	0.50	0.55	0.50	0.55	0.52	0.53
SEM	0.040	0.014	0.034	0.010	0.020	0.013	0.008
Linear Trend	0.7375	0.0728	0.6614	0.6275	0.0963	1.000	0.0691

The findings suggest that BSFL may influence yolk composition in a dynamic manner, rather than consistently, implying that longer studies are needed to observe variations in yolk quality. Factors such as hen age, metabolic adaptation, and protein-lipid balance may also contribute to temporal differences, highlighting a research gap for future investigations into the impact of prolonged BSFL supplementation on yolk lipid composition and fatty acid profiles, key determinants of egg nutritional quality.

Yolk Color

Yolk color, an important factor in egg quality and consumer preference, reflects carotenoid intake and pigment deposition (Shane, 2007). No significant A x B interaction was found ($p>0.05$), but Table 11 shows significant differences in yolk color between housing systems and dietary treatments. Hens in uncaged environments consistently had higher yolk color scores than those in caged systems, with significant differences from week 4 onwards ($p < 0.05$). This aligns with Raphulu et al. (2015) and Gandarillas et al. (2023), who suggested that uncaged hens benefit from greater access to dietary pigments through foraging and a more varied diet, with improved welfare conditions potentially aiding nutrient absorption and pigment metabolism.

Table 11. Yolk Color of layer hens in caged and uncaged systems supplemented with varying levels of BSFL.

FACTORS	WEEK						
	2	4	6	8	10	12	MEAN
SYSTEMS							
Caged	11.69	12.21^{b}	11.53^{b}	11.38^{b}	11.97^{b}	12.32^{b}	11.85
Uncaged	11.81	12.60^{a}	12.03^{a}	12.00^{a}	12.61^{a}	12.76^{a}	12.30
SEM	0.143	0.078	0.087	0.116	0.081	0.066	0.054
p-VALUE	0.5925	0.0053	0.0011	0.0018	<0.0001	0.0003	0.0547
LEVELS							
0%	11.62	12.45	11.99	11.91	12.45	12.54^{c}	12.16^{c}
10%	11.87	12.54	11.70	11.83	12.62	12.74^{b}	12.21^{b}
20%	11.91	12.70	12.37	12.25	12.75	13.00^{a}	12.49^{a}
SEM	0.174	0.135	0.160	0.158	0.151	0.122	0.052
Linear Trend	0.2760	0.2395	0.1458	0.1855	0.2224	0.0385	0.0012

Means within a column having different superscripts are statistically significant ($p<0.05$).

BSFL supplementation significantly influenced yolk color, particularly at week 12 ($p = 0.0385$) and in the overall mean ($p = 0.0012$). Hens receiving 20% BSFL had the highest yolk color scores (12.49), compared to 12.21 in the 10% group and 12.16 in the control group. This supports findings by Morand-Laffargue et al. (2023), who noted that BSFL, rich in xanthophylls like lutein and zeaxanthin, enhances yolk coloration. The combined effect of housing systems and BSFL supplementation indicates that dietary strategies can enhance yolk color, with cage-free systems offering access to natural pigments. This approach may meet consumer demands for natural, deeper yellow-orange yolks and serve as a sustainable alternative to synthetic pigment sources.

4. Conclusion

The study showed that both housing systems and BSFL supplementation levels significantly influenced the voluntary feed intake of hens. Specifically, hens in uncaged environments supplemented with 0% BSFL demonstrated the highest VFI. Regarding the egg quality traits, a significant interaction effect was found only in the egg shape index, where Treatment A (caged, 0% BSFL) exhibited the highest score. Significant effects of the housing systems and supplementation levels were observed in the yolk weight and yolk color, where uncaged birds given 20% BSFL exhibited the highest scores. Only the housing systems exerted significant effects on the egg weight, egg height and width, shell weight and thickness, and albumen weights, showing higher scores in the uncaged group. Meanwhile, no significance was found in the housing system and BSFL supplementation level on the yolk-to-albumen ratio.

Conflict of Interest

The authors declare no conflict of interest.

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